

U.S. Pat. Appl. No. 10/777,790

**In the Specification:**

*Please replace the paragraph beginning on page 1, line 3, as follows:*

This filing is a Divisional of 10/000,776, filed November 30, 2001, which is a continuation-in-part of [US Utility patent application of USSN] U.S. Patent Application No. 09/791,497, filed February 22, 2001, which is a continuation-in-part of USSN [09/568,699] 09/627,897, filed [September 8, 2000] July 27, 2000, and claims benefit from US Provisional Patent Applications USSN 60/146,581, filed July 30, 1999; and USSN 60/147,763, filed August 6, 1999, each of which are incorporated herein by reference in their entirety.

*The paragraph starting on page 2, lines 31-32 and continuing to page 3, lines 1-8, has been amended as follows:*

IL-12 plays a critical role in cell-mediated immunity (Gately et al. (1998) Annu. Rev. Immunol. 16:495-521; Trinchieri (1998) Adv. Immunol. 70:83-243; and Trinchieri (1995) Annu. Rev. Immunol. 13:251-276). Its activities are triggered through a high-affinity receptor complex that gathers two closely related subunits, IL-12R $\beta$ 1 and  $\beta$ 2 (Chua, et al. (1995) J. Immunol. 155:4286-4294; and Preskey et al. (1996b) Proc. Natl. Acad. Sci. USA 93:14002-14007). The p35 subunit has been suggested to bind to [a second] a second soluble cytokine receptor called EBI3 (Devergne, et al. (1997) Proc. Natl. Acad. Sci. USA 94:12041-12046). As yet no biological activity has been reported for the p35-EBI3 pair, however, pairings of IL-12 subunits or IL-12-like subunits with other cytokines may provide information about cell-mediated immunity, e.g. T-cell regulation. Furthermore, the discovery of receptors or receptor subunits for these heteromeric cytokines will also provide information regarding immune regulation.

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*The paragraph on page 3, lines 14-21, has been amended as follows:*

From the foregoing, it is evident that the discovery and development of new lymphokines and their related receptors or receptor subunits, e.g., related to the IL-6/IL-12 cytokine family could contribute to new therapies for a wide range of degenerative or abnormal conditions, which directly or indirectly involve the immune system and/or hematopoietic cells. In particular, the discovery and development of lymphokines which enhance or potentiate the beneficial activities of known lymphokines would be highly advantageous. The present invention provides new interleukin compositions, receptor subunits, and related compounds, and methods for their use.

*The paragraph beginning on page 5, lines 22-32, and continuing to page 6, lines 1-4, has been amended as follows:*

In binding compound embodiments, the compound may have an antigen binding site from an antibody, which specifically binds to a natural IL-D80 polypeptide, wherein: the IL-D80 is a primate protein; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide portion from SEQ ID NO:2, 4, 6, or 8; is raised against a mature IL-D80; is raised to a purified primate IL-D80; is immunoselected; is a polyclonal antibody; binds to a denatured IL-D80; exhibits a Kd of at least 30 [ $M$ ]  $\mu M$ ; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Kits containing binding compounds include those with: a compartment comprising the binding compound; and/or instructions for use or disposal of reagents in the kit. Often the kit is capable of making a qualitative or quantitative analysis. Preferred compositions will comprise: a sterile binding compound; or the binding compound and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

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*The paragraph on page 10, lines 1-6, has been amended as follows:*

The primary activity of IL-27 triggers rapid clonal expansion of antigen specific for naïve human and mouse CD4+ T cells. Moreover, it promotes Th1 polarization and [IFN $\gamma$ ] IFN $\gamma$  production of naïve CD4+ T cells. Mechanistically, these naïve T cells are primed to [response] respond to IL-27 by the production of this composite cytokine by the APCs which interact with these cells. These activities of IL-27 are dependent on simultaneous T cell receptor activation and occur in synergy with IL-12.

*The paragraph on page 15, lines 14-21, has been amended as follows:*

Structural analysis can be applied to this gene, in comparison to the IL-12 family of cytokines. In particular,  $\beta$ -sheet and  $\alpha$ -helix residues can be determined using, e.g., RASMOL program, see Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-White (1995) [TIBS] Trends in Biol. Sci. 20:374-376; and Gronenberg, et al. (1991) Protein Engineering 4:263-269. Preferred residues for substitutions include the surface exposed residues which would be predicted to interact with receptor. Other residues which should conserve function will be conservative substitutions, particularly at [position] positions far from the surface exposed residues.

*The paragraph on page 22, lines 7-14, has been amended as follows:*

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.), Academic Press, New York; and particularly in Kohler and Milstein (1975) [in] Nature 256:495-497, which discusses one method of generating monoclonal antibodies.

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*The paragraph on page 40, lines 9-22, has been amended as follows:*

FACS analysis of detectably stained IL-D80, EBI3, and WSX-1/TCCR molecules led to the finding that these molecules are components in a receptor subunit/ligand complex. Specifically, the composite cytokine of E-tagged hIL-D80 (hIL-D80E) and F-tagged [(Flag-tagged)] FLAG-tagged hEBI3 (FhEBI3) binds to Baf3 cells expressing an F-tagged version of WSX-1/TCCR, also referred to as hNR30. The cells were stained using anti-E mAb and a PE-conjugated anti-mouse Fab<sub>2</sub> fragment. Co-immunoprecipitation experiments also indicated that hIL-27 could be immunoprecipitated with R-tagged (RGSH<sub>6</sub>-tagged) soluble WSX-1/TCCR (shNR30R). Alternatively, shNR30R could be co-immunoprecipitated in the presence of hIL-D80E/FhEBI3 complex using anti-E or anti-F mAbs. These experiments establish that WSX-1/TCCR is a receptor component of the IL-27 composite cytokine. Recent evidence shows that disrupting the WSX-1/TCCR gene in mice results in lowered expression of [IFN<sub>γ</sub>] IFN-γ, which is a critical cytokine in the mediation of pro-inflammatory functions. These mice were unable to mount a Th1 response (See, e.g., Chen, et al. (2000) Nature 407:916-920[.]) .

*The paragraph beginning on page 47, lines 31-32 and continuing to page 48, lines 1-6, has been amended as follows:*

cDNAs encoding full length human and mouse IL-D80 were cloned into the [pCDM8-etag] pCDM8-E-tag vector via HindIII-Xhol (h/mp28-E). EBI3: human and mouse EBI3 were cloned into pME18S-Ig vector via EcoRI/Xhol (h/mEBI3-Ig) and the mature portion of human EBI3 into [pFlagCMV-1] p-FLAG-CMV-1 vector via HindIII-NotI (F-hEBI3). One chain fusions EBI3/p28: HindIII-XbaI fragments were generated encoding the mature part of human or mouse EBI3, followed by the synthetic linker GSGSGGSGGSGSGK (SEQ ID NO:13) and by the mature coding sequence of human or mouse IL-D80 via HindIII-NotI. Fragments were inserted into pFLAG-CMV-1 (Sigma, St. Louis, MO) using HindIII-NotI sites.

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*The paragraph on page 48, lines 7-14, has been amended as follows:*

WSX-1/TCCR: the protrypsin leader peptide and the [flagtag] FLAG-tag encoding part of [pFlagCMV-1] p-FLAG-CMV-1 vector were deleted by PCR, instead an RGSH<sub>6</sub>-tag was introduced via Sall/SmaI (pCMV-1-RGSH<sub>6</sub>); the cDNA encoding the extracellular part of human WSX-1 was cloned into this vector via HindIII-Sall (soluble hWSX-1-R). In general restriction sites were introduced through the respectively used PCR primers and cDNA was [ampified] amplified using standard PCR protocols. Proteins were produced via transient expression in HEK293T cells. For experiments requiring pure proteins purification was performed by affinity chromatography using the respective protein tags.

*The paragraph on page 48, lines 17-24, has been amended as follows:*

1x10<sup>6</sup> HEK293T cells were transiently transfected with a total amount of 5 µg plasmid DNA (control vector, expression vectors encoding h/m p28-E, E-hEBI3 and mEBI3-Ig, or respective combinations). Cells were cultured for 24 hr after transfection, then metabolically labeled for 16 hr with 50 µCi/ml Pro-mix L-[<sup>35</sup>S] *in vitro* cell labeling mix (Amersham Pharmacia, Piscataway, NJ) in cysteine/methionine free MEM. Proteins were precipitated from supernatants with either [anti-Flag] anti-FLAG M2 agarose (Sigma, St. Louis, MO), with [anti-etag] anti-E-tag mAb bound to protein G [sepharose] Sepharose® (Amersham Pharmacia), or with protein A [sepharose] Sepharose® (Amersham Pharmacia).--

*The paragraph on page 48, lines 27-32, has been amended as follows:*

The mature part of human and mouse WSX-1 was cloned into pMX vector via HindIII-NotI, then a sequence encoding the protrypsin leader peptide fused to a [flag] FLAG epitope was cloned into the vector in frame and 5' of WSX-1 via BamHI-

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HindIII (E-h/mWSX-1). Retrovirus obtained by transfection of BOSC23 cells was used to infect parental Ba/F3 cells and cell surface expression of the desired proteins was monitored using a [flag-PE] FLAG-PE-staining in FACS analysis.

*The paragraph on page 51, lines 18-25, has been amended as follows:*

Appropriate host cells were transiently transfected with empty vectors or expression vectors encoding hIL-D80E (E=E-tagged) and/or FhEBI3 [(F=Flag-tagged)] (F = FLAG-tagged). Cells were cultured to 24 hrs. and then metabolically labeled for 16 hrs with 50  $\mu$ Ci/ml PRO-MIX L-[<sup>35</sup>S] in vitro cell labeling mix (Amersham Pharmacia) in cysteine/methionine free MEM cell culture media. Proteins were precipitated from 300 mL supernatant with either the anti-His5 mAb or anti-E or anti-F mAb. The IL-12R like subunit, WSX-1/TCCR, was also detectably labeled with RGSH<sub>6</sub>-tag (shNR30R) and immunoprecipitated as above.

*The paragraph beginning on page 51, lines 28-32, and continuing to page 52, lines 1-4, has been amended as follows:*

Purified labeled IL-27 composite cytokine or IL-27-WSX-1/TCCR complex were run on a nonreducing 10% NUPAGE gel in MES running buffer (Novex, San Diego, CA). Appropriate lanes were excised, reduced in sample buffer containing DTT, laid horizontally on two-well 10% gels, and run reduced in a second dimension. One gel was silver stained (Daiichi, Tokyo, Japan) while the other was blotted to a PVDF membrane and developed using appropriate mAbs. It was found that hIL-80E could be co-immunoprecipitated with shNR30R in the presence of FhEBI3 using the anti-His<sub>6</sub> mAb. Alternatively, shNR30R could be immunoprecipitated in the presence of hIL-80E and FhEBI3 using the anti-E mAb or anti-F mAb.

*The paragraph on page 52, lines 8-17, has been amended as follows:*

CD4+CD45RB<sup>high</sup> or CD4+CD45RB<sup>low</sup> T cell subsets were purified from the spleen and mesenteric lymph nodes of >6 month old IL-10-/ C57/B6 N12 mice as

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described (Davidson et al., 1998) (1998) *J. Immunol.* 161:3143-3149). Cells were fractionated into CD4+CD45RB<sup>high</sup> and CD4+CD45RB<sup>low</sup> cell populations by two color sorting on a FACSTAR plus (Becton Dickinson, San Jose, CA). All populations were >99% pure upon reanalysis. CD4+CD45RB<sup>high</sup> or CD4+CD45RB<sup>low</sup> were put into a proliferation assay with plate bound anti-CD3 (145.2C11) stimulation as described (Davidson et al., 1998) (1998) *J. Immunol.* 161:3143-3149). Additions to the growth media included anti-IL-2 [Mab] mAb (JES6-1A12) 100 µg/ml, and cytokines as indicated. Cells were incubated for 5 days in a humidified chamber (37°C, 5% CO<sub>2</sub>) with [<sup>3</sup>H]TdR (Amersham) added at a final concentration of 1 µCi/well for the last 24h of incubation.

*The paragraph beginning on page 52, lines 25-31, and continuing to page 53, lines 1-2, has been amended as follows:*

FACS purified CD45RA and CD45RO T cells (purity > 99%) were cultured at a density of 4X10<sup>4</sup> cells/well in a 96-well plate previously coated with anti-CD3 antibody at 10 µg/ml and soluble anti-CD28 at 1 µg/ml with or without IL-26/EBI3. Anti-hIL-2 [Mab] mAb 17H12 and anti-hIL-2R [Mab] mAb B-B10 (Diaclone, Besancon, France) were added at 10 µg/ml where indicated. IL-27 was also able to induce proliferation of FACS sorted human CD45RA naive T cells isolated from peripheral blood mononuclear cells (PBMC). Similar to the results with mouse naive T cells, IL-27 induced strong proliferation of CD3/CD28 naive T cells in the presence of anti-IL-2. This response was enhanced by the addition of IL-12. No response was seen with IL-27 treated CD45RO memory cells.

*The paragraph on page 54, lines 13-28, has been amended as follows:*

Because of the relationship between IL-27 and the IL-6/IL-12 family, the search for the signaling receptors was concentrated on this family. Members of this family were introduced into BaF3 cells and tested for binding to IL-27. Of the receptors tested only Ba/F3 cells expressing the orphan cytokine receptor WSX-1/TCCR (see, e.g., Sprecher, et al. (1998) Biochem. Biophys. Res. Comm. 246:82-90; and Chen, et al.

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(2000) *Nature* 407:916-920) showed binding to tagged IL-27. BaF3 cells infected with retroviral constructs expressing either E-tagged human or mouse WSX-1 cDNA (E-hWSX-1 or E-mWSX-1) showed cellular staining using [anti-Flag] anti-FLAG mAb. Cells expressing E-hWSX-1 were then incubated with either hEBI3-Ig alone or with coexpressed hIL-D80-E and EBI3-Ig for [two] two hours. Heterodimeric IL-D80/EBI3 bound to WSX-1 while EBI3-Ig itself showed no detectable binding. Similarly, only the combination of mIL-D80-E and mEBI3-Ig provided a detectable interaction with mWSX-1-expressing BaF3 cells, whereas the two individual proteins were not able to do so. Incubation of independently expressed mIL-D80-E and mEBI3-Ig with E-mWSX-1 expressing BaF3 cells also led to cellular staining. Untransfected control cells were not stained by IL-D80/EBI3, demonstrating the specificity of the observed interactions.

*The paragraph beginning on page 54, lines 29-32, and continuing to page 55, lines 1-9, has been amended as follows:*

These results were confirmed by co-immunoprecipitation experiments using a soluble extracellular form of hWSX-1 with a C-terminal RSGH<sub>6</sub>-tag (R). Proteins from supernatants of transiently transfected HEK293T cells containing E-hEBI3 or coexpressed hILD80-E / E-hEBI3 were immunoprecipitated using either [Flag] FLAG M2-agarose, protein G [sepharose-coupled anti-etag] Sepharose®-coupled anti-E-tag mAb (Amersham Pharmacia, Piscataway, NJ) or protein G [sepharose-coupled] Sepharose®-coupled anti-H<sub>5</sub> mAb. The primary [pricipitates] precipitates were washed and then incubated with HEK293T cell supernatants containing shWSX-1-R. Secondary precipitates were [seperated] separated by SDS-PAGE and subjected to western blot. [Precipitated] Precipitated proteins were visualized by [ECL] enhanced chemiluminescence (ECL) using antibodies against the respective protein tags. Only when all three proteins were present (hIL-D80-E, E-hEBI3 and shWSX-1-R), immunoprecipitation of one protein brought down both other components independently of the immunoprecipitating antibody used. The same co-immunoprecipitation experiment using the respective mouse orthologues had similar results.